Kishimoto *et al.* ("Kishimoto"), either or both references in view of Gross *et al.* ("Gross"), and Farkas *et al.* ("Farkas"). Applicants respectfully traverse, for reasons of record and further in view of the following comments.

The presently pending claims are directed to a method of preventing or treating acute pancreatitis by administration of an antibody which binds to the IL-6 receptor. Logically, to find the claims obvious, the prior art would have to show two things:

1. That IL-6 is causative of pancreatitis, in that IL-6 leads to, or enhances pathology, and is not merely a marker or a secondary symptom of the disease; and
2. That an antibody which binds to the IL-6 receptor is effective in preventing or treating pancreatitis, and does not cause secondary pathologies that actually enhance the disease or create other diseases.

Although the second point is by no means resolved, the present impasse largely turns on the first point of whether the person of ordinary skill at the time of filing would believe that the evidence shows that IL-6 is causative of pancreatitis. The PTO position is that the evidence is sufficiently strong that a person of ordinary skill would believe that IL-6 is causative of acute pancreatitis. Applicants agree that the evidence shows that IL-6 is an indicator of pancreatitis, along with other indicators, but do not agree that it rises to the level of demonstrating causation. Indeed, if there were sufficient evidence, as the PTO asserts, then such a link would have been made explicit in the art. That the art does not make such an explicit statement indicates that a causal link had not been demonstrated, or that there was sufficient uncertainty such that a person of ordinary skill in the art would not have had a reasonable expectation that pancreatitis could be successfully treated with an antibody that binds to the IL-6 receptor. Given that antibodies to IL-6 and the IL-6 receptor were already known several years before the filing date of the present application, there was ample opportunity to demonstrate the role of IL-6 in pancreatitis. However, the earliest evidence demonstrating that IL-6 is causative of pancreatitis, and not symptomatic, is the present application, which shows that blocking IL-6 by targeting the IL-6 receptor treats pancreatitis. The PTO's assertions of obviousness rely on impermissible hindsight reconstruction of the evidence to arrive at the present invention. Not only is there impermissible hindsight reconstruction, but there are also additional defects in the arguments presented by the PTO.

First is the reliance on Farkas. Farkas is concerned with the link between proinflammatory cytokines and *multiple-organ failure*, not proinflammatory cytokines and *pancreatitis*. The Farkas abstract recites "[p]roinflammatory cytokines may play a role in the development of multi-organ failure during pancreatitis. . . We conclude that cytokines, such as TNF and IL-6, may contribute to the vasogenic brain formation during acute pancreatitis." Thus, Farkas shows that IL-6 causes increased blood-brain barrier permeability, *a secondary complication of pancreatitis*. Farkas is not, however, dispositive or even relevant to the role of IL-6 in *pancreatitis*. It is striking that Farkas, who was actively investigating the role of IL-6 and TNF in the secondary complications of pancreatitis, is silent on the role of IL-6 or TNF in the primary disease, especially since targeting IL-6 would treat *both* pancreatitis and pancreatic encephalopathy.

The PTO makes much of Table 1 of Farkas which "clearly shows that serum IL-6 levels were greatly elevated at 4, 24 and 48 hours, compared to at 0 hours, becoming more elevated as the acute pancreatitis progressed." Office Action at page 3. This data is clearly relevant to the pancreatic encephalopathy, but *teaches away* from causation of pancreatitis because one would expect that a causative agent would *precede* and not *follow* the disease. Table 1 is consistent with IL-6 being a symptom of pancreatitis, rather than a cause.

In fact, Farkas, on page 149, left column, line 10 states "[h]owever, TNF seems to be a more important mediator in that respect, because the induction of TNF preceded that of IL-6, and the peak of serum TNF level was seen in the first 24h during which the increased blood-brain barrier permeability was detected." This means that an increased expression of IL-6 is observed a long time after generation of pancreatitis, while that of TNF is earlier than IL-6. Because TNF is known to induce IL-6, the observations of Farkas are more consistent with TNF as the causative agent of pancreatitis than IL-6, with IL-6 merely being symptomatic of increased TNF production.

Regarding Gross, the PTO states "Gross specifically teaches that IL6 is not only associated with, but predictive of the severity of acute pancreatitis." Office Action at page 3. Again, Applicants note that correlation does not equal causation. Indeed, Figure 1 of Gross shows IL-6 causing an acute phase liver response. It does not link IL-6 to pancreatic inflammation or tissue destruction. Thus Gross is consistent with IL-6 as an *indicator* of pancreatitis (albeit not as good as PMN-elastate, page 525, the left column), and an *indicator*

of PMN/leukocyte activation, but does not go so far as to conclude that it is causative of pancreatitis.

Regarding Farkas and Gross together, the PTO asserts that "[i]t simply begs credulity that these teachings would not suggest to the person of ordinary skill in the art that reduction of IL-6 levels in patients with acute pancreatitis would be beneficial." That the causative link is so obvious and clear to the PTO now, but was apparently not to those of ordinary skill in the art at the time, suggests that the PTO is engaging in impermissible hindsight reconstruction. Indeed, it begs credulity that if such a link were known or obvious at the time, that a researcher would not have made such a link explicit and claim credit for the discovery.

The PTO asserts that IL-6 "would been expected by the person of ordinary skill in the art to have a causative effect in acute pancreatitis because of its well-known property of being an inflammatory cytokine (for example, see Farkas' abstract)." Again, this would appear to be impermissible hindsight reconstruction of the data, and one that is contradicted by the notable absence of such an explicit link being made by those of skill in the art. Moreover, this statement assumes a greater knowledge of the pathology of pancreatitis and the functions of IL-6 than was present at the time of filing. As to the disease, the PTO has not shown that cytokine-induced inflammation occupied a central role in pancreatitis. As to the cytokines, many different cytokines may be found associated with disease, including inflammatory disease. Moreover, there are many different diseases with an inflammatory component, but the relative roles of the different inflammatory cytokines differ in each disease. Therefore, a role in pancreatitis does not follow merely because IL-6 is inflammatory. Even if IL-6 was previously shown to be at least a contributing factor in pancreatitis, there is no teaching or suggestion that its role is sufficiently important that blocking of IL-6 activity would be as effective as it is. The PTO has not established a reasonable expectation that the disease could be treated by binding to the IL-6 receptor.

Three references provide relevant insight into the present dispute.

The Knulst reference (previously provided) is regarded by the PTO as irrelevant to the present claims because Knulst concerns acute graft v. host disease (GVHD) and not pancreatitis. However, the PTO is required to consider evidence of unsuccessful attempts or negative data in the consideration of obviousness, and Knulst presents such data. The abstract of Knulst makes clear "a significant rise in serum levels of IL-6, TNF-α and IFN-γ

was found" in mice with GVHD. GVHD has an inflammatory component and IL-6, TNF-α and IFN-γ are all inflammatory cytokines. However, monoclonal antibody antagonists against IL-6, TNF-α and IFN-γ failed to treat GVHD. Therefore, Knulst shows that despite strong correlation between IL-6 and disease, these cytokines are not necessarily causative and monoclonal antibody antagonists are not therapeutic. By extension, the correlation between IL-6 and pancreatitis is not sufficient to show that IL-6 is causative of pancreatitis or that antibody antagonists would be therapeutic.

The second reference, Murata *et al.* "Possible implications of cytokines in the pathophysiology of acute pancreatitis, *Saishin Igaku* 47(11):49-56, 1992, is provided as an attachment, along with an English translation thereof (hereinafter the English translation will be referred to as "Murata"). Also provided as an attachment is the third reference, the abstract of Guice *et al.* "Anti-tumor necrosis factor antibody augments edema formation in caerulin-induced acute pancreatitis," *J. Surg. Res.* 51: 495-499 (1991) ("Guice") which was cited in the Murata reference.

Figure 5 of Murata identifies cytokines as a result, and not a cause, of acute pancreatitis. This is consistent with the other data showing correlation, but not showing IL-6 as a cause of pancreatitis. In addition, TNF-α, IL-1 and IL-8 are identified as central to pathology, with IL-6 being peripheral and causing only acute phase reactants. Therefore, the person of ordinary skill would believe that the relevance of IL-6 to pancreatitis is speculative and, at best, peripheral. The person of ordinary skill, rather, would target TNF, IL-1 or IL-8. However, the results obtained are contrary to expectations.

> administration of anti-TNF antibody in cerulein-induced pancreatitis augmented not only pancreatic edema but also pulmonary lesions. The inhibition of the TNF action by the pretreatment prevents from transmitting the abnormality called cerulein-induced pancreatitis to the body's defensive system. In other words, when rats themselves try to complete the inflammatory reaction by their own defense system, the first step signal, TNF cannot transmit its signal to the next one. As the result, anti-inflammatory reactions cannot be taken place so that pancreatic inflammation becomes severe, and pancreatic inflammation itself is aggravated more by severe local tissue lesions. Thus it is considered that pulmonary lesions were developed by a cytokine network without a TNF-mediated pathway.

Murata also describes the complicated networks of cytokines in pancreatitis:

> Many cytokines overlap in their biological activities. Furthermore, one cytokine controls the induction of another kind of cytokine and modifies its activity, thereby the body's defense response to an invasion has been controlled complicatedly and elaborately. Therefore, it may be meaningless that the pathophysiology of SIRS is explained only by the involvement of one or two kinds of cytokines.

Id. at page 12, lines 2 to 9. Further, at page 13, lines 3 to 7, that "[i]t is important that the induction of cytokines by an invasion such as pancreatitis is the body' normal defense response. Thus the inhibition of all the cytokine reactions may conversely aggravate inflammation."

Therefore, although TNF is correlated with pancreatitis (and induces IL-6), administration of an anti-TNF antibody *worsens* pancreatitis rather than treating it. This result strongly *teaches away* from the manipulation of cytokine levels as a treatment of pancreatitis. The person of ordinary skill therefore, would consider (a) that IL-6 is of peripheral relevance to pathology and (b) that targeting IL-6 may seriously worsen, rather than assist treatment, especially given the complex network of cytokines.

In summary, therefore, the prior art does show an association between IL-6 and pancreatitis. However, this art does not show that this correlation equals causation, and certainly not the direction of causation asserted by the PTO. Rather the art shows significant reasons to believe that IL-6 is only one of several indicators; is a symptom, not a cause, of pancreatitis; and is, at most, peripheral to disease pathology. Secondly, the art does not show that administration of an antibody against the IL-6 receptor would treat or prevent pancreatitis, given both that the role of IL-6 was not known, and that such treatment may not work or even exacerbate the disease. The only rationale offered to support the assertions of obviousness is one based on hindsight reconstruction of the evidence which is not only impermissible, but is contradicted by the failure of those in the art at the time to make the same connection now asserted as obvious by the PTO.

Accordingly, the art, as a whole, does not render obvious the claimed invention. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 103 is respectfully requested.

CONCLUSION

In view of the foregoing remarks, Applicants respectfully submit that all of the pending claims are now in condition for allowance. An early notice to this effect is earnestly solicited. If there are any questions regarding the application, the Examiner is invited to contact the undersigned at the number below.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing or a credit card payment form being unsigned, providing incorrect information resulting in a rejected credit card transaction, or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorize payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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Sarshin Igaku

発生機序と病態

膵炎とサイトカイン

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要　旨

急性膵炎，中でも重症急性膵炎の病態は，膵局所だけでなく遠隔重要臓器の障害いわゆる多臓器障害として捉えることができる．近年，この多臓器障害発症機序にサイトカインが深く関与していることが解明されつつある．しかしTNF，IL-1といった炎症性サイトカインが，急性膵炎の病態にどのように関わっているかは，今のところ明らかではない．

急性膵炎患者の血中サイトカイン濃度の測定から，我々は重症膵炎でもこれら炎症性サイトカインが関与している可能性を指摘してきた．サイトカインと多臓器障害を結ぶものとして活性化好中球があり，生体に起こった侵襲の指標あるいは信号としてサイトカインが誘導され，それがどのような機序で臓器障害に至るかについて，急性膵炎を生体に起こった一つの侵襲と考えて，その関係を解明して行きたい．

重症急性膵炎の病態は，脳障害，循環障害，呼吸障害，肝障害，腎障害，DIC，消化管障害，といういわゆる多臓器障害（MODS：multiple organ dysfunction syndrome；あるいはMOF）の病態とまさに一致する．近年このMODSの病態にサイトカインが関与していることが次々に明らかにされつつあるが，本稿では，膵炎の発症からMODSへと進展して重症膵炎に陥って行く病態にサイトカインが如何に関与しているかを，我々の知見をもとに推察してみたい．

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キーワード：急性膵炎，炎症性サイトカイン，多臓器障害，生体反応

I．多臓器障害からみた重症膵炎

生体に過度の侵襲が加わると様々な反応が起こる．これらをsystemic inflammatory response syndrome（SIRS）として捉えると，図1[1]のように，感染の有無，あるいは敗血症の有無に関わらず，これまでのMOF（MODS）と考えていた病態として理解される．SIRSとは，種々の疾患により，（1）体温38℃以上あるいは36℃以下，（2）脈拍90/min以上，（3）呼吸回数20回/min以上，あるいは$PaCO_2$ 32mmHg以下，（4）白血球数12000/mm^3以上，あるいは4000/mm^3以下，あるいは幼若球10%以上になった状態，

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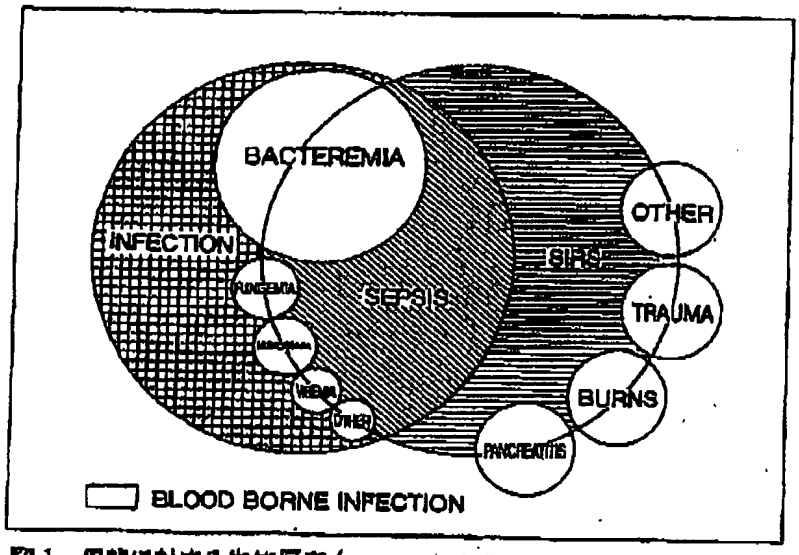

図1 侵襲に対する生体反応（syetemic inflammatory response syndrome）と sepsis，感染との関係（文献1より）

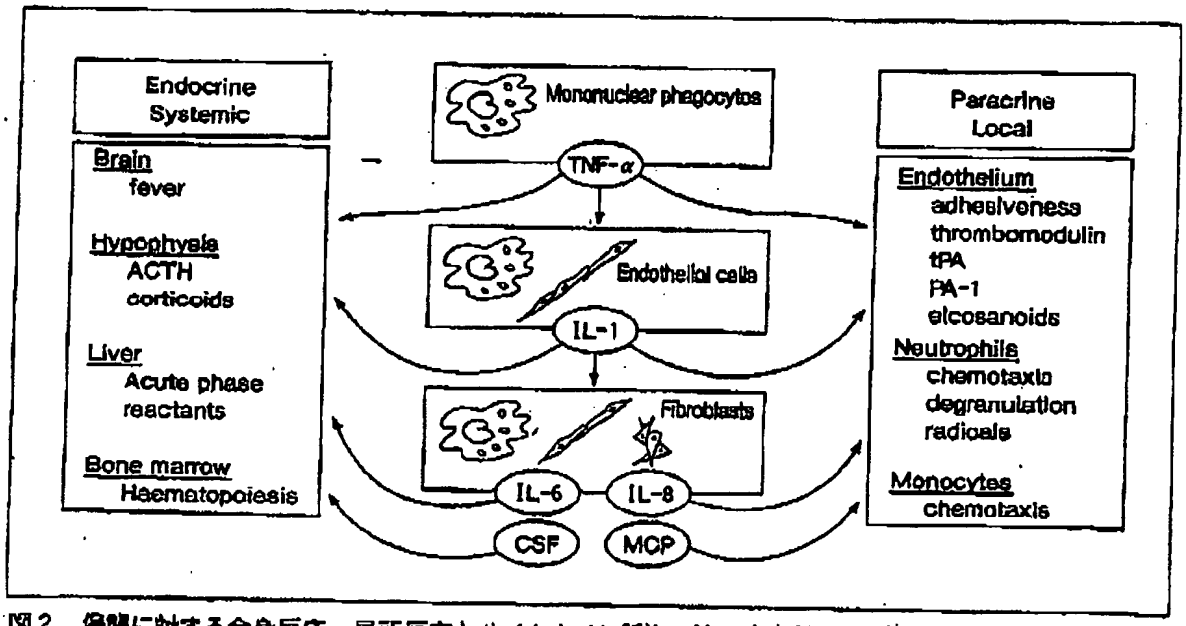

図2 侵襲に対する全身反応，局所反応とサイトカイン誘導の流れ（文献2より）

つまりこれまでは bacteremia，sepsis，septic shock，MODS という流れの中で論じてきていた病態が，必ずしも感染という存在なくしても当てはまるということである．そしてこの SIRS をもたらす key mediator としてサイトカイン，特に炎症性サイトカインと呼ばれる TNF，IL-1，IL-6，IL-8 の四つが注目されている．

これらのサイトカインを中心に，侵襲に対する局所反応，全身反応の流れを見ると，図2[2]のように考えることができる．侵襲により単球から TNF が誘導され，この TNF が

IL-1 を誘導する．この両者はお互いの誘導を更に起こすと共に，IL-6，IL-8，CSF，MCP（MCAF）といった二番手のサイトカインを誘導する．生体反応の一つである発熱は，TNF，IL-1，IL-6 で起こるし，肝細胞での急性相反応物質は IL-6 を中心に誘導されることが知られている．CSF は骨髄に働いて，血球を動員し，IL-8 は好中球の遊走，活性化を促進し，MCP はマクロファージを遊走活性化して，これらすべてで一連の炎症反応系を形成することができる．また，局所では，血管内皮細胞や血小板，好中球などに働いて，接着因子の発現やプロスタグランジン，活性酸素の産生にも関与している．つまり，今まで我々が侵襲に対する生体反応として捉えてきたことを，サイトカイン発現の流れの中で見いだすことができる．

重症急性膵炎も先に述べたように SIRS の一つである．即ち，その病態にこれらサイトカインが深く関わっていることが強く示唆される．図3は，実際の急性膵炎患者の血中 IL-6 値を示したものである．重症度は厚生省難治性膵疾患研究班の基準によるものだが，重症急性膵炎でその初期の IL-6 の値は他の2群に比べて有意に高い．血中 IL-6 がこのように高いということは，その前に TNF や IL-1 も上昇していると考えられるが，今のところヒトの血中 TNF，IL-1 の値を正確に測定することはできない．これは，TNF や IL-1 の血中存在様式によるものであろうが，免疫学的測定ではキットによる差や施設間のバラツキが大きく，推測するしかない．IL-6 と同時に IL-8 も，図4に示すように重症膵炎で上昇することが分かった．このことは我々の施設だけでなく，Leser ら[2]，Gross ら[3]によっても確認されている．

以上のことから，MODS を伴う重症急性膵炎では，cytokinemia（高サイトカイン血症）が存在すること，つまり重症膵炎とサイトカインとの関与が強く支持される．

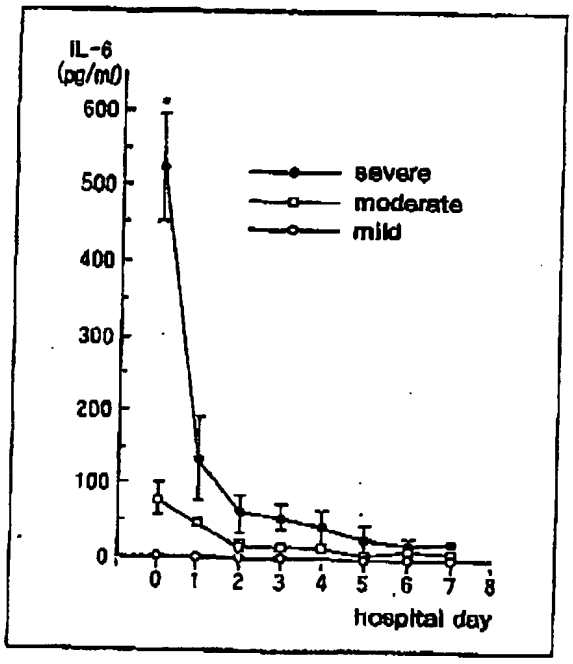

図3　急性膵炎患者の血中 IL-6 の経日的変動

II. サイトカインと多臓器障害

ARDS をはじめとする MODS の病態に関する研究は古くから行われており，特に好中球の関与が知られている．好中球の活性化は補体や，アラキドン酸代謝産物，バクテリア菌体成分などで起こるが，この好中球活性化にもサイトカインが深く関与している[4]．我々も *in vitro* の系で，好中球がサイトカインで活性化されて正常細胞を障害することを明らかにしている[1]．更に，好中球はサイトカインによって遊走することや，サイトカインの刺激で活性化好中球からエラスターゼが放出され細胞障害に重要な役割を担っていることから，侵襲時にみられる MODS の発症に，サイトカインとそれによって活性化される好中球が重要な位置にいると考えている．また，臨床例でも，手術という生体に加わった侵襲後の血中サイトカイン量と好中球の関係を調べてみると，術後早期に IL-6 が上昇

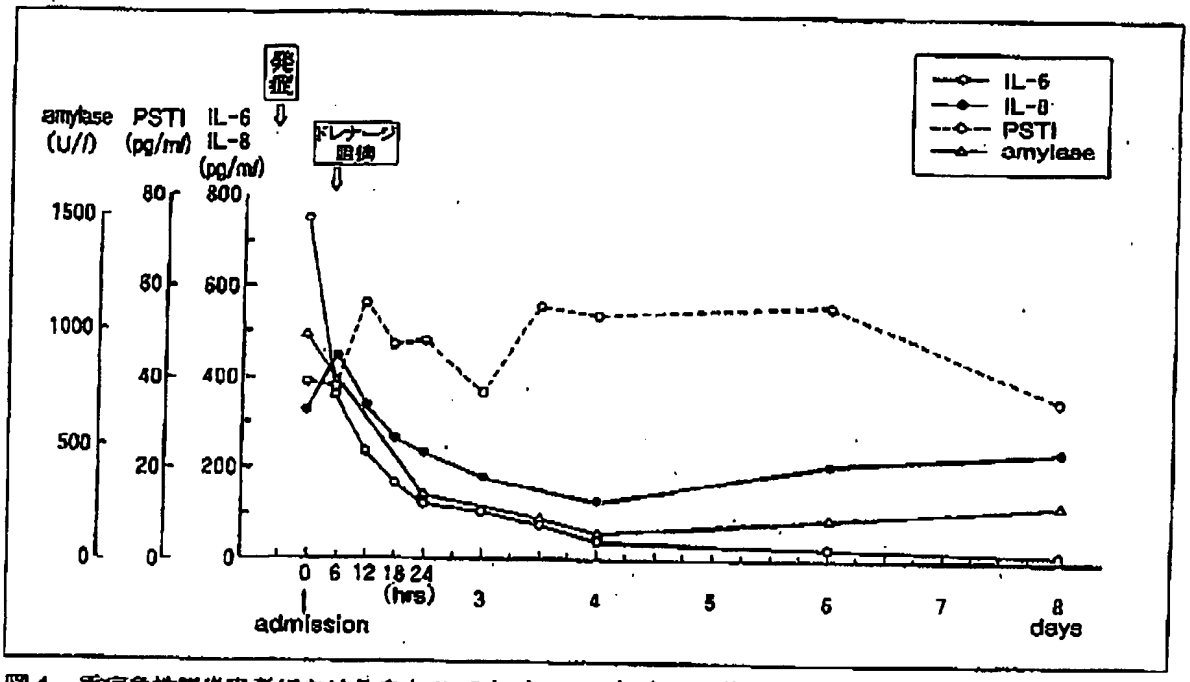

図4　重症急性膵炎患者における血中 IL-6（○），IL-8（●）の変動

し，そのピーク値は好中球の活性化の指標である好中球エラスターゼのピーク値と有意の正の相関を示すことも明らかにしている[9]．更に，術後合併症を併発した群で，IL-6 の値，好中球エラスターゼの値が有意に高いことから，生体に対する侵襲の大きいほど，つまり組織障害が大きいほどサイトカインが大量に誘導され，そのサイトカインによって好中球がより強く活性化されていることが分かる[9]．IL-8 も IL-6 と同様に sepsis などで早期に血中に多く誘導され，病態と深く関わっていることが報告されている[10][11]．

SIRS という反応をもたらした侵襲により血中に多量のサイトカインが誘導され，それによって好中球が活性化される機構が徐々にではあるが明らかにされつつある．

Ⅲ．膵炎とサイトカイン

これまで急性膵炎に伴う ARDS などの臓器障害は，膵から逸脱した活性化プロテアーゼや PL-A_2 などによりもたらされると考えられてきたが[12]，生体には多量の蛋白分解酵素阻害物質があることや，臓器障害発症時期と膵そのものの炎症極期とのずれなどから，我々は重症膵炎と MODS を結ぶ mediator として別のものを考えてきた．膵炎にみられる呼吸障害は，肺胞の血管透過性亢進や間質の浮腫，炎症細胞浸潤など ARDS そのものである．実験的に動物に急性膵炎を作成すると，時にこの ARDS と同じ変化が肺にみられる[13]．

ARDS はこれまでの研究から好中球が関与していることは明らかで，実際 実験膵炎モデルでの活性化好中球による肺障害も報告されている[14]．何故，肺が最も好中球にやられ易いのであろうか．それは，肺が好中球を多く含む重要臓器であり[15]，同時に多量のサイトカインを分泌でき得る臓器でもあるからである[16]．これは，肺という臓器が常に外界に対して防御機構を準備しておかなければならない臓器の一つであることから，合目的な反応であると言える．

生体に膵炎という一つの侵襲が起こったとき，生体は先に述べたようにサイトカインを誘導して防御機構を発動させる．急性膵炎を他の侵襲，例えば熱傷や多発外傷などと同じように考えると，好中球の活性化が単に膵炎特有の現象ではなく，侵襲に対する生体反応の一つとして捉えられる[17]．言い換えると，サイトカインを炎症の“alarm hormone”と考えれば，膵炎発症という生体の異常をTNF，IL-1が伝達し，IL-6，IL-8が続いて誘導され，生体防御網が形成される．膵そのものは活性化した膵酵素で組織障害が起こり，更に活性酸素などが加わって膵局所の細胞，内皮細胞なども障害を受ける．この障害を受けた膵局所でTNFやIL-1が誘導されると，障害の程度に応じてTNF，IL-1の量も変わり，続いて誘導されるIL-6，IL-8，CSFの量もコントロールされるはずである．通常炎症は局所への炎症細胞浸潤，異物処理，組織融解，線維化などで終焉するはずである．

では，この機構のどこに異常が起これば，膵炎からMODSといった全身障害がもたらされるのであろうか．言い換えれば，誘導されるサイトカインの量なのか，タイミングなのかという疑問である．

既に述べたように重症急性膵炎患者では高サイトカイン血症がある．つまり，大量のサイトカインが重症膵炎では誘導されている．我々はこの問題の解決のために，実験膵炎モデルにおけるサイトカインの関与を研究している．ラットにセルレインを使って急性浮腫性膵炎を作ることができる．この方法が動物に最も侵襲を加えないやり方である．他の重症膵炎モデルでは，実験そのものの侵襲が強すぎて，動物の内因性サイトカインを強く誘導してしまうからである．ラットセルレイン膵炎でも時として，膵の脂肪壊死や，細胞浸潤，肺障害がみられるが，これは内因性にラットのサイトカインが誘導されてこのような反応を起こしたと考えられる．

動物にTNFやIL-1（ヒトのリコンビナント）を投与すると，ショックや肺障害，肝障害を作ることができるが[18]，セルレイン膵炎もラットにとっては侵襲であり，TNF，IL-1が内因性に誘導されるはずである．この内因性サイトカインの量を凌駕するほどの量のサイトカイン（recombinant human TNF＋IL-1）をセルレイン膵炎モデルに追加投与してみたところ，セルレイン膵炎では見られない強い肺障害や，死亡例ができることが分かった．これまで，セルレイン膵炎だけで肺に好中球が浸潤する例はみられていたが，ヒトの好中球遊走因子であるIL-8に対するラットのcounterpartはまだ見つかっておらず，ラットのモデルでIL-8の関与を証明することはできない．TNF，IL-1がラットの内因性の好中球遊走活性化因子としてのサイトカインを誘導していることは，十分考えられる．

このように実験膵炎モデルでのサイトカインの関与はまだ推測の域を出ないが，多量に加えたTNFあるいはIL-1が，セルレインで起こる浮腫性膵炎という病理病態を修飾し，全身障害を伴うような膵炎を発症させ得ると考えられる．一方，TNFに関して次のような面白い報告がある．セルレイン膵炎に抗TNF抗体を投与しておくと，膵の浮腫が強くなったばかりか，肺障害も強くなったというものである[19]．前投与でTNFの作用を抑えてしまうと，セルレイン膵炎という異常を伝達できなくなり，本来はラット自身が反応して炎症反応を終わらせようとしているところを，初めのステップであるTNFの信号が次に伝わらず，抗炎症反応が起こらずに膵の炎症がひどくなり，局所の組織障害が強くなって膵の炎症そのものが更に悪化し，それによってTNFを介さないサイトカインネットワークが発動して肺障害がもたらされたと

考えられる．

以上のことをシェーマにすると，図5のようになる．急性膵炎という膵局所の組織障害は，TNF，IL-1，IL-6，IL-8といった炎症性サイトカインを誘導する．今のところ臨床的には，IL-6濃度と，それによって誘導される急性相反応物質を正確に測定できる．ここに当然，エンドトキシン，補体，血小板，PAFなども深く関与するが，これらは，いわゆる侵襲に対するSIRSという生体反応を惹起する．重症膵炎でIL-6，IL-8が高値をとることから，TNF，IL-1の誘導も異常に起こったと考えられる．TNF，IL-1，IL-8は膵局所だけでなく，遠隔重要臓器，例えば肺における好中球と内皮細胞の反応を刺激し，活性化した好中球がその重要臓器の細胞を障害する．障害を受けた組織から更にサイトカインが誘導されると，循環している好中球がまた活性化され，この攻撃に参加する．この病態が，SIRSからMODS（従来のsepsis syndromeからMOF）へと変わって行くメカニズムと，著者らは考えている．

Ⅳ．ま　と　め

現在ヒトでサイトカインと呼ばれている物質は約30数種類ある．サイトカインの多くは分子量が15-30kDaの糖蛋白で，各々に特有のレセプターを介してその細胞に働く．サイトカインは単球やリンパ球だけでなく，内皮細胞や線維芽細胞，上皮細胞，好中球そのものなどから分泌されることが分かってきた．また，多くのサイトカインの生物活性が重複しており，更に一つのサイトカインは他のサイトカインの誘導をコントロールしたり，その作用を修飾することにより侵襲に対する生体防御反応を複雑かつ巧妙に支配している．従って，SIRSという病態を一つや三つのサイトカインだけで説明することは無意味かも

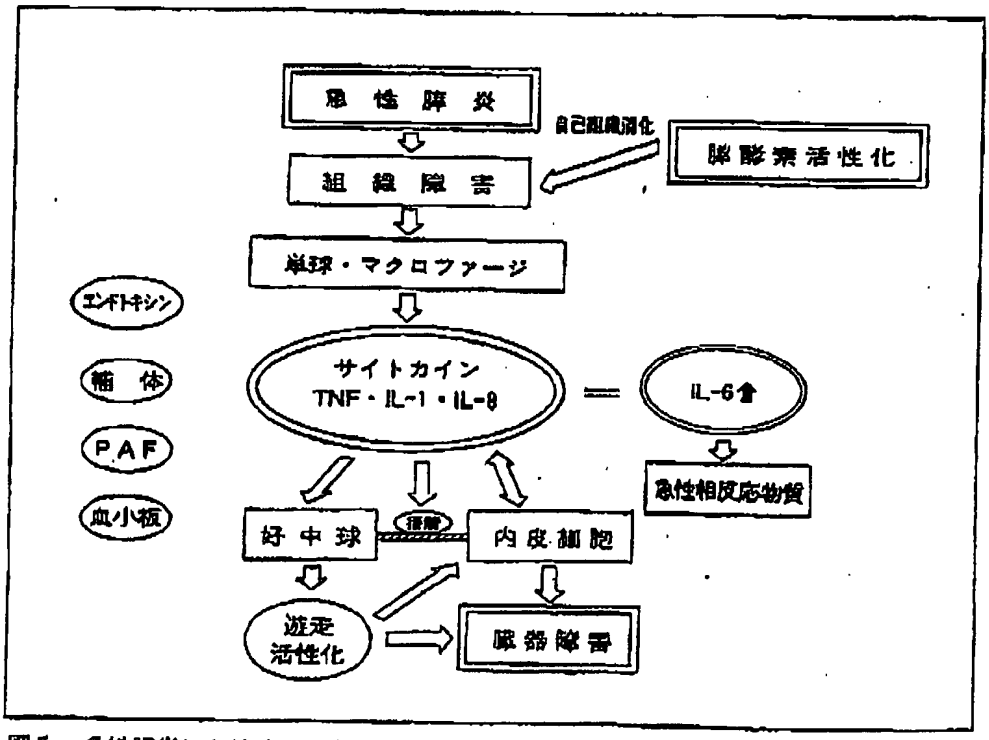

図5　急性膵炎におけるサイトカインの関与と臓器障害発症機序（仮説）

しれない．しかし，脾炎のみならずSIRSという病態の研究を行うのは，あたかも擦れた糸を1本1本解いて行くようなものである．やっと今その糸のはしが見えてきたと言える．

重症膵炎の治療は，MODS（MOF）の治療そのものである．重症膵炎という病態にこれまで述べてきたようにTNFやIL-1といった炎症性サイトカインが関与しているとすれば，その治療法の主眼もこれまでの抗膵酵素療法ではなく，今MODSやARDSの治療に応用されようとしている方法を取るべきである[20]．ただ，幸いなことに，これまで用いてきた膵炎の治療薬のうち，蛋白分解酵素阻害剤と呼ばれるものは，単に膵の蛋白分解酵素だけでなく，好中球エラスターゼを阻害したり，補体の活性化を抑えたり，まさしくSIRSあるいはMODSの治療の一部であったことである．

これからは更に一歩手前であるサイトカインの誘導あるいは，その作用の抑制を目的として，サイトカインレセプターアンタゴニストやサイトカイン抗体，更に可溶性レセプターを用いた新しい治療法が開発されるであろう．その時重要なことは，膵炎のみならず侵襲によって誘導されるサイトカインは本来は正常な生体反応であり，そのサイトカイン反応をすべて抑えてしまうと，逆に炎症を悪化させる可能性があるということであろう．そのためにも，重症膵炎の臨床例あるいは動物実験モデルでのサイトカインの生理的量や反応，分泌されるサイトカインの種類やタイミングなどまだまだ多くの未解決の問題が残っている．

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Possible Implication of Cytokines in the Pathophysiology of Acute Pancreatitis

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Development mechanism and pathophysiology

Possible Implication of Cytokines in the Pathophysiology of Acute Pancreatitis

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Summary

The pathophysiology of acute pancreatitis, especially severe acute pancreatitis can be understood as the lesions in distant important organs as well as pancreas itself, so-called multiple organ dysfunction syndrome. Recently, it has been elucidated that cytokines are deeply involved in the development mechanism of multiple organ dysfunction syndrome. It is, however, still unknown how inflammatory cytokines such as TNF and IL-1 are involved in the pathophysiology of acute pancreatitis.

Based on the assay of blood cytokine concentrations in patients with acute pancreatitis, we have pointed out that

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these inflammatory cytokines probably involves even in the development of severe pancreatitis. Activated neutrophil is a mediator for the development of multiple organ dysfunction syndrome by cytokines, which are induced as an indicator or sign of an invasion produced in the body. We try to elucidate how multiple organ dysfunction syndrome is produced in acute pancreatitis, an invasion in the body.

Key words: acute pancreatitis, inflammatory cytokines, multiple organ dysfunction syndrome, body's response

The pathophysiology of severe acute pancreatitis just coincides with that of so-called multiple organ dysfunction syndrome(MODS or MOF) consisting of cerebral lesions, circulatory disorders, respiratory disorders, hepatic lesions, renal lesions, DIC and gastrointestinal disorders. It is now getting to be clarified more and more that cytokines are involved in the pathophysiology of MODS. In this article, based on our findings, we try to elucidate how cytokines are involved in the advancement from the onset of pancreatitis to severe acute pancreatitis, namely MODS.

I. Severe pancreatitis in multiple organ dysfunction syndrome

Excess invasion induces various responses in the body. When these responses are classified as systemic inflammatory

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response syndrome (SIRS), as shown in Fig. 1 [1], they can be understood as the pathophysiology of MOF (MODS) irrespective of the presence or absence of infection or sepsis. SIRS can be defined as the following conditions produced by various diseases: (1) body temperature; ≥ 38°C or ≤ 36°C, (2) pulse; ≥ 90/min, (3) respiratory rate; ≥ 20 /min or $PaCO_3$: ≤ 32 mmHg and (4) white blood cell count: ≥ 12000/mm^2 or ≤ 4000/mm^3, or immature neutrophil; ≥ 10%. In other words, the pathophysiology that has been considered as the serial events of bacteremia, sepsis, septic shock and MODS can be understandable even without the involvement of infection. As the key mediators for the development of SIRS, cytokines, especially four inflammatory cytokines, TNF, IL-1, IL-6 and IL-8, have been paid an attention.

The flow of cytokines in local and systemic responses to the invasions can be considered as shown in Fig. 2 [2]. An invasion induces TNF production in monocytes and the TNF induces IL-1 production. These both cytokines induce the production of the secondary cytokines such as IL-6, IL-8, CSF and MCP (MCAF) as well as their own production each other. Fever, a biological response, is produced by TNF, IL-1 and IL-6, and it has been known that acute phase reactants in the liver cell are induced mainly by IL-6. CSF acts on the bone marrow to stimulate haematopoiesis, IL-8 stimulates chemotaxis and activation of neutrophils, and MCP stimulates chemotaxis of

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macrophages. All these reactions can make up a series of events in the inflammatory response system. These cytokines also act locally on vascular endothelial cells, platelets and neutrophils to express adhesive factors, and to produce prostaglandins and active oxygens. In other words, the events that we have understood as the biological responses to an invasion can be found in the flow of the cytokine expression.

Severe acute pancreatitis is classified as SIRS as described previously. It is, therefore, strongly suggested that cytokines are deeply involved in the pathophysiology of severe acute pancreatitis. Fig. 3 shows the IL-6 values in patients with acute pancreatitis. The severity was determined according to standard criteria presented by the investigation research team on intractable pancreatic diseases of the Ministry of Health, Labour and Welfare. The IL-6 values at the early stage of severe acute pancreatitis were significantly higher than those of other two groups, moderate and mild pancreatitis groups. It is considered that such high blood IL-6 level could be caused by the increases in the TNF and IL-1 levels. Right now, TNF and IL-1 in human blood, however, cannot be determined accurately. The difficulty in the determination of these factors is probably due to the form of their existence in blood. There are large differences in the immunological assay among assay kits and among institutes. Therefore, we have just to conjecture that TNF and IL-1 levels

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were probably increased before the IL-6 elevation. As shown in Fig. 4, it was found that IL-8 together with IL-6 was also increased in severe pancreatitis. This phenomenon has been confirmed not only by our institute but also by Leser et al. [3] and Gross et al.[4]

These results indicate that cytokinemia exists in severe acute pancreatitis with MODS, which strongly supports the involvement of cytokines in the development of severe panoreatitis.

II. Cytokines and MODS

The research on the pathophysiology of MODS such as ARDS has been performed for a long time and it has been known that especially neutrophils are involved in the pathophysiology of MODS. Neutrophils are activated by complements, arachidonic acid metabolites, bacterial constituents, etc. and cytokines are deeply involved also in the activation of neutrophils.[5][6] We have also clarified in an *in vitro* system that neutrophils are activated by cytokines to attack on normal cells. [7] Furthermore, chemotaxis of neutrophils is induced by cytokines and cytokines release esterases from the activated neutrophils, which plays an important role in the production of cell damage. Therefore, it has been considered that cytokines and neutrophils activated by cytokines occupy an important position in the development of MODS induced by an invasion.

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We examined the relationship between the blood cytokine level after the surgical invasion and the behavior of neutrophils in clinical field and have clarified that the IL-6 level was increased at the early phase after surgery and that there was a positive relationship between the peak of the IL-6 level and the peak of neutrophil esterase, a marker of neutrophil activation. [8] In the group having the postoperative complications, the values of IL-6 and neutrophil esterase were significantly high. Therefore, it can be understood that when the larger invasion occurs to the body, in other words, the larger cell damage occurs, the larger cytokines are induced to activate neutrophils more strongly. [9] It has been reported that a large amount of IL-8 is also induced in blood at the early phase of the onset of sepsis etc. as in the case of IL-6 and that the induction is deeply involved in the pathogenesis of sepsis etc. [10] [11]

It has been clarified, though gradually, how neutrophils are activated by cytokines, a large amount of which is induced in blood by an invasion causing the response called SIRS.

III. Pancreatitis and cytokines

Until now, it was considered that organ lesions such as ARDS were caused by activated proteases and PL-A_2 released from the pancreas. [12] We have considered that some different factors might be involved in the connection of severe

pancreatitis and MODS, because there are a large amount of protease inhibitors in the body and there is a difference between the time of the onset of MODS and the time of the inflammatory peak in the pancreas itself. The respiratory lesion in pancreatitis is ARDS itself such as the alveolar vascular permeability enhancement, interstitional edema and inflammatory cell infiltration. In the experimental acute pancreatitis model animals, the same change as that in ARDS can be observed in the lung sometimes. [13]

From the research performed until now, it becomes clear that neutrophils are involved in the development of ARDS. In fact, it has been reported that pulmonary lesions are produced by activated neutrophils in an experimental pancreatitis model. [14]. Why is the lung most easily attacked by neutrophils? The answer is that the lung is an important organ containing plenty of neutrophils [15] and at the same time an organ secreting plenty of cytokines. [16] This can be said to be a reasonable response, because the lung is one of the organs that have always to provide a defense mechanism against an external attack.

The onset of pancreatitis, an invasion in the body, makes the defense mechanism active by inducing cytokines as previously described. When acute pancreatitis is considered as the same as the invasions of burns and multiple trauma, the activation of neutrophils is not a characteristic phenomenon of pancreatitis but can be understood as our body's response

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against an invasion.[17] In other words, if cytokines are considered as "alarm hormone" of inflammation, the body's abnormality produced by the onset of pancreatitis is transmitted by TNF and IL-1, and IL-6 and IL-8 are successively induced to form the body's defense network. In the pancreas itself, the tissue lesions are produced by the activated pancreatic enzymes, and the pancreatic local cells and endothelial cells receive a lesion by the involvement of active oxygens etc. When TNF and IL-1 are induced at the damaged pancreas itself, the amount of TNF and IL-1 induced changes dependent on the degree of the lesion and the amount of IL-6, IL-8 and CSF successively induced should be also controlled. The ordinary inflammatory event should come to an end after a series of reactions such as local inflammatory cell infiltration, elimination of foreign body, histolysis and fibrogenesis.

Where is the abnormality point in this mechanism for the development of the systemic lesions such as MODS produced by pancreatitis? In other words, which is the key point for the development, the amount of cytokines induced or the timing of cytokine induction?

As previously described, cytokinemia exists in patients with severe acute pancreatitis. Namely, a large amount of cytokines is induced in severe pancreatitis. In order to solve this problem, we have studied on the involvement of cytokines

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in the development of pancreatitis in an experimental pancreatitis model. Acute edematic pancreatitis can be induced in rats by cerulein. This method produces least invasion against animals. In other severe pancreatitis models, the experimental invasion itself is too severe to strongly induce endogenous cytokines in animals. The cerulein-induced pancreatitis in rats, however, shows sometimes fat necrosis and cellular infiltration in the pancreas and pulmonary lesions. It is considered that such responses were produced by the induction of endogenous cytokines in rats.

Administration of TNF and IL-1 (human recombinant) to animals can produce shock, pulmonary lesions and hepatic lesions.[19] Cerulein-induced pancreatitis is also an invasion against rats so that TNF and IL-1 should be induced endogenously. When cytokines (recombinant human TNF + IL-1) were administered to the cerulein-induced pancreatitis model animals at the dose of far excess amount of endogenous cytokines, it was found that severe pulmonary lesions and mortality, which can not be produced in cerulein-induced pancreatitis, were produced. Until now, neutrophil infiltration into the lung has been observed only in some cerulein-induced pancreatitis animal models. The counterpart of human IL-8, a neutrophil chemotactic factor, has not been found in rats yet. Therefore, it cannot be proved whether IL-8 is involved in the development of pancreatitis in rats. It is, however, fully presumed that

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TNF and IL-1 induce cytokines as the endogenous neutrophil chemotactic factors of the rats.

Although the involvement of cytokines in the experimental pancreatitis animal model is still within the presumption, it is considered that a large amount of TNF or IL-1 administered can develop pancreatitis with systemic organ lesions by modifying the pathophysiology of edematic pancreatitis induced by cerulein. On the other hand, concerning TNF, there is an interesting paper, in which it was reported that administration of anti-TNF antibody in cerulein-induced pancreatitis augmented not only pancreatic edema but also pulmonary lesions. [19] The inhibition of the TNF action by the pretreatment prevents from transmitting the abnormality called cerulein-induced pancreatitis to the body's defensive system. In other words, when rats themselves try to complete the inflammatory reaction by their own defense system, the first step signal, TNF cannot transmit its signal to the next one. As the result, anti-inflammatory reactions cannot be taken place so that pancreatic inflammation becomes severe, and pancreatic inflammation itself is aggravated more by severe local tissue lesions. Thus it is considered that pulmonary lesions were developed by a cytokine network without a TNF-mediated pathway.

The above-mentioned theory is summarized as a scheme shown in Fig. 5. Local tissue lesions in the pancreas called

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acute pancreatitis induce inflammatory cytokines such as TNF, IL-1, IL-6 and IL-8. Right now, IL-6 and acute phase reactants induced by IL-6 can be accurately determined in clinical field. As a matter of course, endotoxin, complements, platelets, PAF, etc. are deeply involved in the events of acute pancreatitis. These factors induce the body's response against invasion called SIRS. Since the values of IL-6 and IL-8 were high in severe pancreatitis, it is considered that abnormal induction of TNF and IL-1 also occurred. TNF, IL-1 and IL-8 damage not only local pancreas but also distant important organs. For example, these cytokines stimulate the reaction between neutrophils and endothelial cells in the lung and the activated neutrophils damage the important organ cells. Cytokines are induced more from the damaged tissues and circulating neutrophils are activated again to join in this attack. We have considered that this pathophysiology is a mechanism, by which SIRS advances to MODS (from sepsis syndrome to MOF in former definition).

IV. Summary

There are now about thirty several kinds of substances called cytokine in human. Most of cytokines are glycoproteins with a molecular weight of 15 to 30 KDa and act on a cell through a specific receptor for each cytokine. It has been clarified that cytokines are secreted from endothelial cells, fibroblast

cells, epithelial cells and neutrophils themselves as well as from monocytes and lymphocytes. Many cytokines overlap in their biological activities. Furthermore, one cytokine controls the induction of another kind of cytokine and modifies its activity, thereby the body's defense response to an invasion has been controlled complicatedly and elaborately. Therefore, it may be meaningless that the pathophysiology of SIRS is explained only by the involvement of one or two kinds of cytokines. The research on the pathophysiology of SIRS as well as pancreatitis, however, looks like to unfasten twisted strings one by one. It can be said that the end of the twisted strings can be just found now.

The therapy of severe pancreatitis is that for MODS (MOF) itself. If inflammatory cytokines such as TNF and IL-1 are involved in the pathophysiology of severe pancreatitis as described above, the therapeutic method, which is now tried to apply to the treatment of MODS and ARDS, rather than the existing anti-pancreatic enzyme therapy should be employed for the treatment of severe pancreatitis.[20] Luckily, however, pancreatic protease inhibitors, the existing therapeutic drugs for pancreatitis inhibit not only pancreatic protease activity but also neutrophil esterase activity and the activation of complements, which are just a part of the therapy for SIRS and MODS.

In future, in order to inhibit the induction of cytokines

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and their activities. a novel therapeutic method using cytokine receptor antagonists, anti-cytokine antibody or soluble cytokine receptors will be developed. It is important that the induction of cytokines by an invasion such as pancreatitis is the body's normal defense response. Thus the inhibition of all the cytokine reactions may conversely aggravate inflammation. Thus there are still many unsolved problems such as physiological amount and reactions of cytokines, type of cytokines secreted and timing of secretion of cytokines in the clinical cases of severe pancreatitis and the experimental severe pancreatitis animal model.

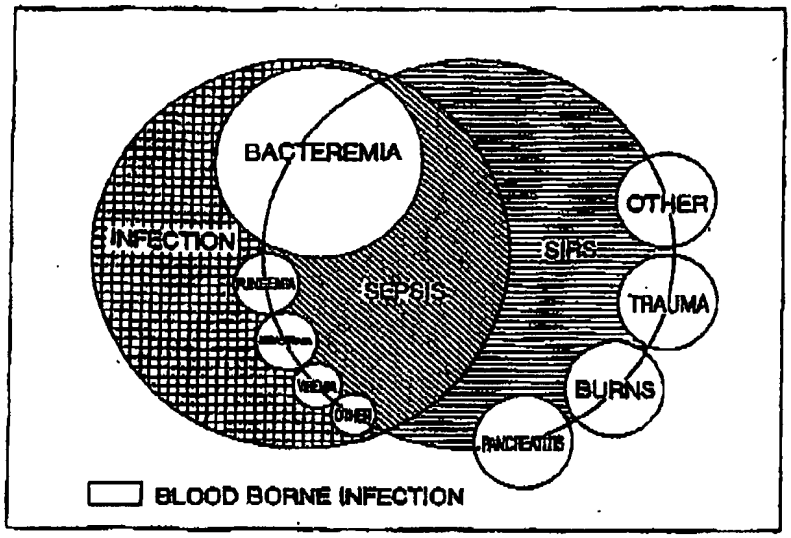


Fig. 1 Correlation of systemic inflammatory response syndrome, sepsis and infection (reproduced from reference [1])

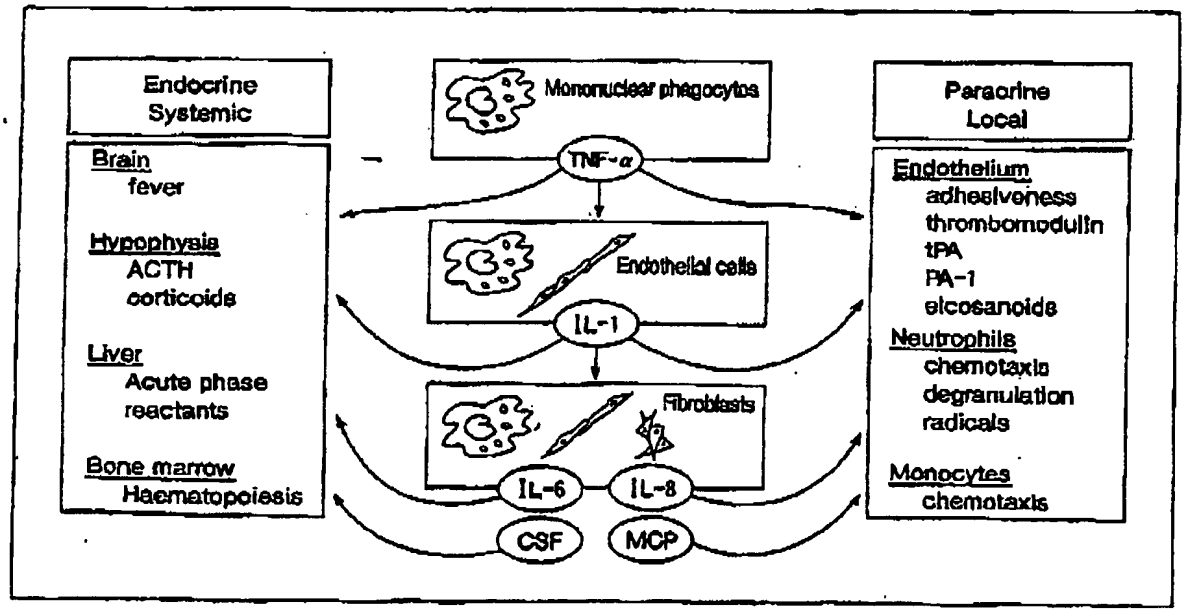


Fig. 2 Flow of cytokine induction in systemic and local response to invasion (reproduced from reference [2])

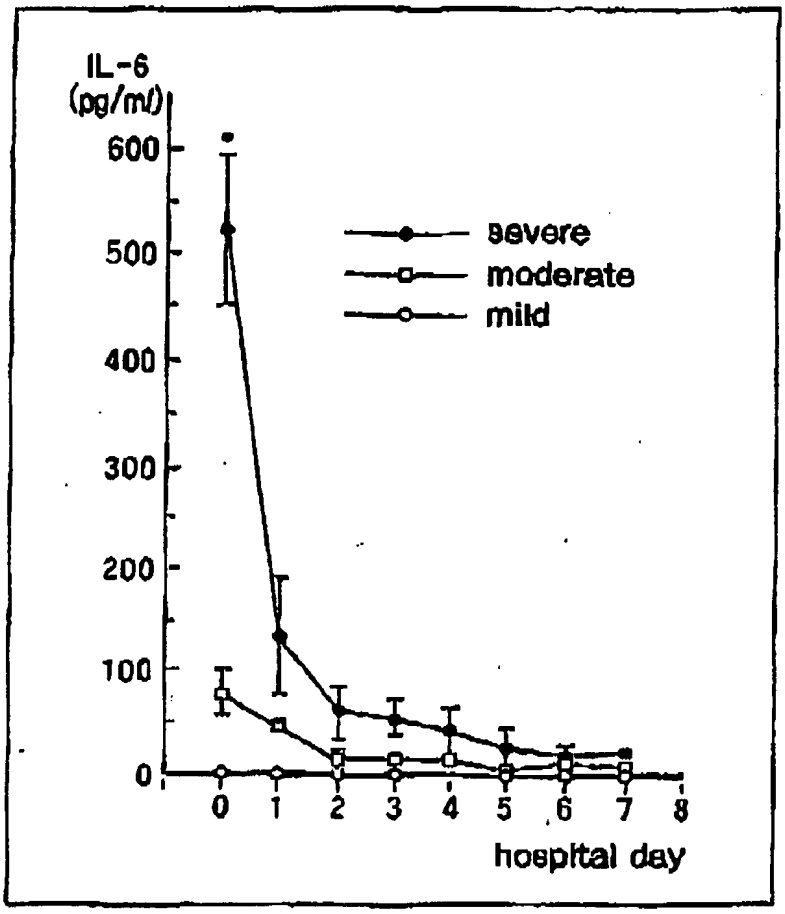


Fig. 3 Daily change of blood IL-6 level in patients with acute pancreatitis

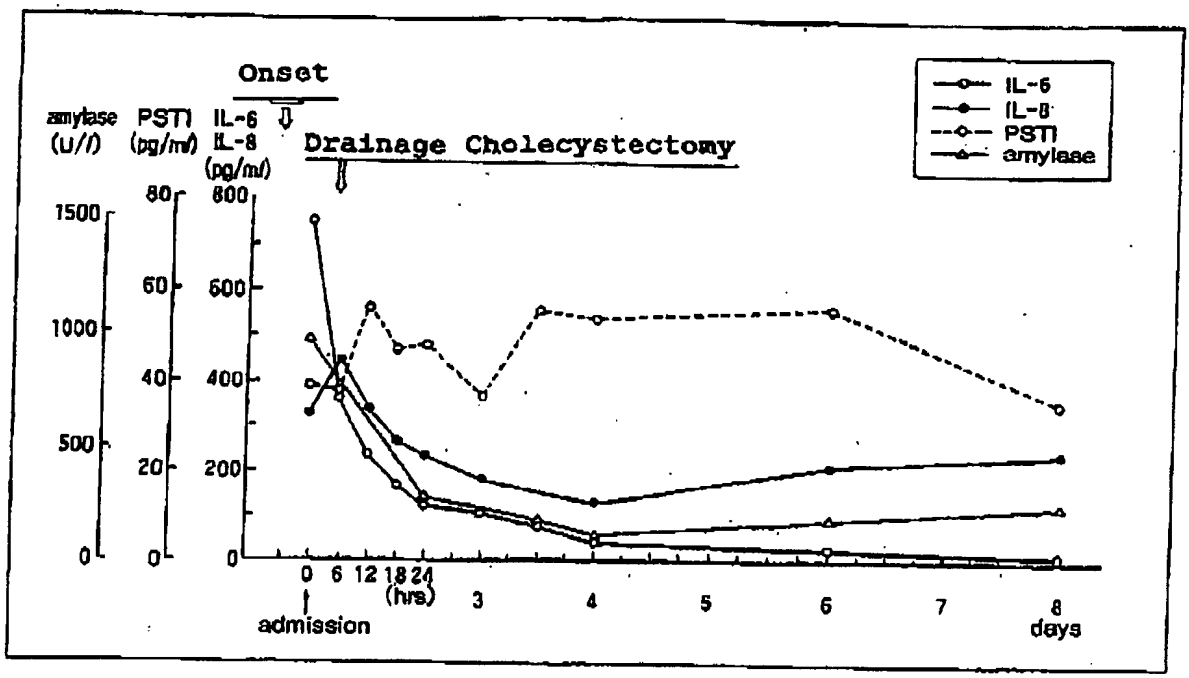


Fig. 4 Change of blood IL-6 (○) and IL-8 (●) levels in patients with severe pancreatitis

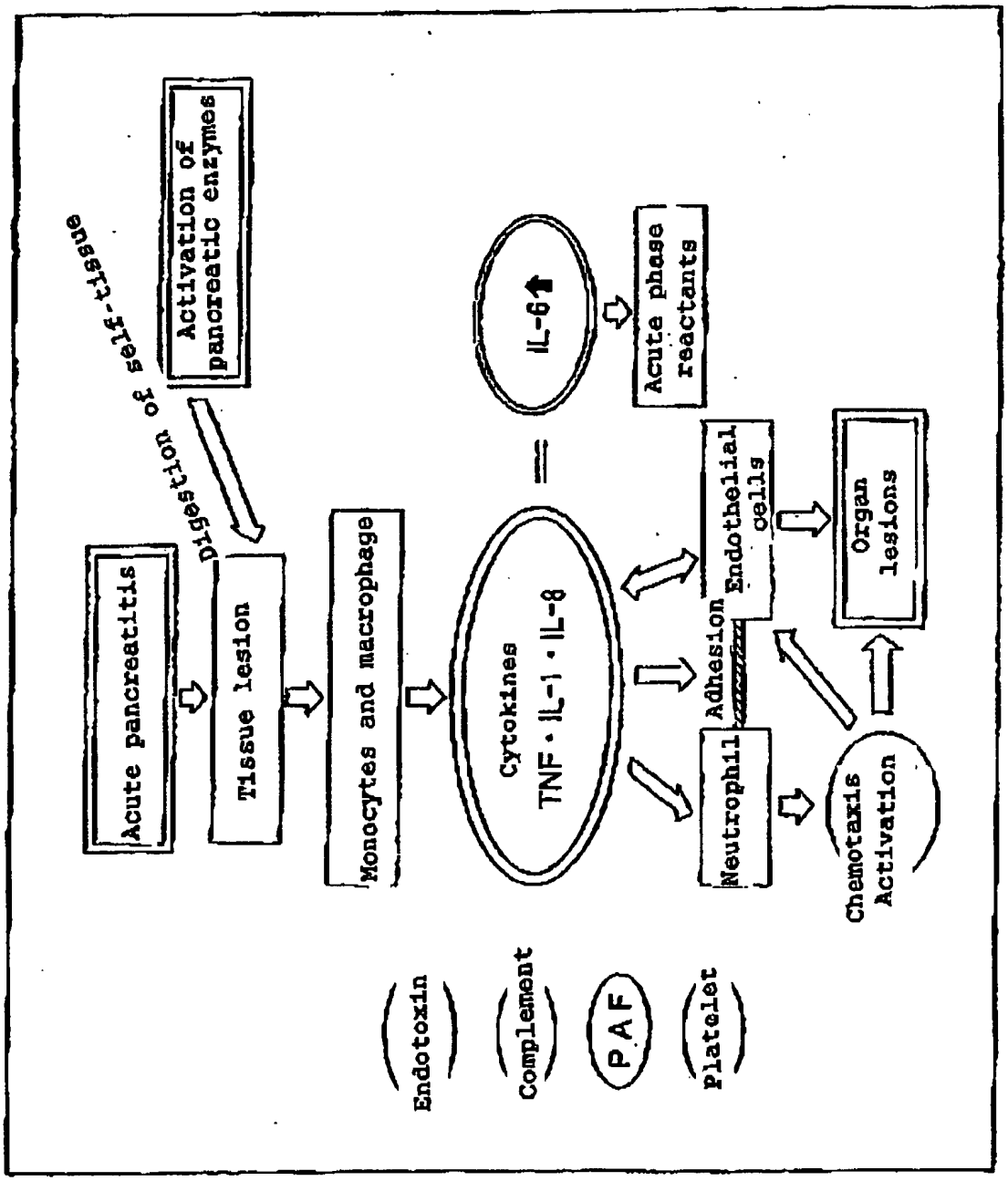
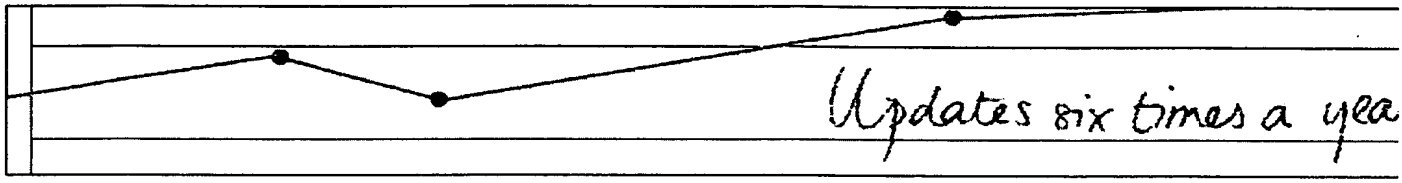


Fig. 5 Involvement of cytokines and mechanism of development of organ lesions in acute pancreatitis (hypothesis)



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Anti-tumor necrosis factor antibody augments edema formation in caerulein-induced acute pancreatitis[*1, , *2]

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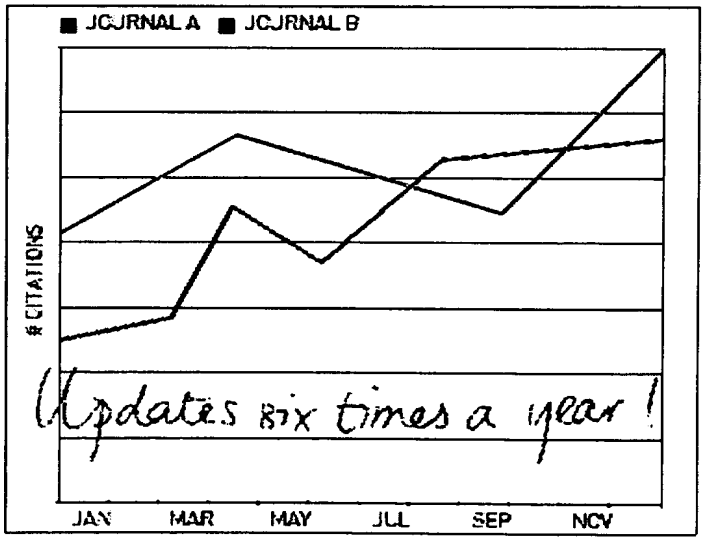

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Abstract

The pathogenesis of acute pancreatitis is

incompletely defined, but the outcome is determined in part by an acute inflammatory process.

Pancreatitis-associated inflammation appears to play a role in the local retroperitoneal injury as well as in the associated dysfunction of remote organs such as the lung. Tumor necrosis factor (TNF) appears to be a proximal mediator of the inflammatory response. In this study, anti-TNF antibody was administered to rats with caerulein-induced pancreatitis to determine if the observed increases in pancreatic and pulmonary microvascular permeability were related to plasma TNF activity. In contrast to the expected findings, blockade of TNF activity was found to increase the amount of edema formation in both the pulmonary and pancreatic microvascular beds. The mechanism is not known; however, blockade of TNF-induced down regulation of phagocytic cell activity, ablation of TNF-dependent feedback inhibition of other cytokines, failure of induction of endogenous antioxidant systems, or inactivation of the TNF control of microvascular tone are all possible explanations. This is potentially an important observation as clinical strategies are now being developed to modify the inflammatory response in ways presumed advantageous to an injured host.

Article Outline

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